PB83-145243

Experimental Infection of Mongrel Dogs with 'Giardia lamblia' Cysts and Cultured Trophozoites

Case Western Reserve Univ.
Cleveland, OH

Prepared for

Health Effects Research Lab.
Cincinnati, OH

13 Jul 81

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1. REPORT NO. EPA-600/J-82-088	2. JOURNAL ARTICLE	3. PB83-145243
4. TITLE AND SUBTITLE JOURNAL ARTICLE: Experimental Infection of Mongrel Dogs with Giardia lamblia Cysts and Cultured Trophozoites		5. REPORT DATE 1982 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Erik L. Hewlett, John S. Andrews, Juanita Ruffier and Frank W. Schaefer, III		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Div. of Geographic Medicine, Dept. of Med., Case Western Reserve University School of Medicine; & Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH 45268; J.S. Andrews' present address is CDC, Atlanta,GA		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Public Health Service (Grant #AI-15351) and the Rockefeller Foundation.		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/11

15. SUPPLEMENTARY NOTES

Journal of Infectious Diseases, 145(1):89-93, January 1982

16. ABSTRACT

In light of recent epidemiologic data implicating wild and domestic animals in the transmission of giardiasis, a study was undertaken to determine whether mongrel dogs could be infected with Giardia lamblia. After careful screening by stool examination (a minimum of six stools examined per week for three weeks), dogs were channenged orally with G. lamblia cysts from human stool or trophozoites from an axenic culture. Three of eight dogs became infected with cysts, and two of three became infected with trophozoites. Latency periods and the course of cyst excretion were similar to those observed in humans, but the dogs were not clinically ill. These data support the concept of interspecies transmission of Giardia and demonstrate that infection can be transmitted by the trophozoite form as well as by cysts.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) unclassified	21. NO. OF PAGES 6
	20. SECURITY CLASS (This page) unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA-600/J-82-088
JOURNAL ARTICLE

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 145, NO. 1 • JANUARY 1982 89-93

Experimental Infection of Mongrel Dogs with *Giardia lamblia* Cysts and Cultured Trophozoites

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In light of recent epidemiologic data implicating wild and domestic animals in the transmission of giardiasis, a study was undertaken to determine whether mongrel dogs could be infected with *Giardia lamblia*. After careful screening by stool examination (a minimum of six stools examined per week for three weeks), dogs were challenged orally with *G. lamblia* cysts from human stool or trophozoites from an axenic culture. Three of eight dogs became infected with cysts, and two of three became infected with trophozoites. Latency periods and the course of cyst excretion were similar to those observed in humans, but the dogs were not clinically ill. These data support the concept of interspecies transmission of *Giardia* and demonstrate that infection can be transmitted by the trophozoite form as well as by cysts.

In early studies of the host specificity of *Giardia* species, there was disagreement over the transmission capabilities of the parasite between species. Because *Giardia* speciation was based on the animal host of origin, many species were identified without careful study to determine host range. Hegner [1] proposed rigid host specificity for *Giardia* species, a proposition which he later revised with his demonstration of apparent transmission of *Giardia* organisms from humans to rats [1A]. At present, in the absence of immunologic or other satisfactory criteria for speciation, it appears that there are several *Giardia* species, including *Giardia muris* and *Giardia lamblia* (previously, *Giardia duodenalis*), that are capable of infecting various mammalian species [2].

Received for publication April 6, 1981, and in revised form July 13, 1981.

This work was supported in part by grant no. AI-15351 from the U.S. Public Health Service and by the Rockefeller Foundation.

We thank Jane Myers, Nan Gregor, Robert Voight, and Dr. Sherman Sachs for their assistance with the care of the dogs, Drs. A. A. F. Mahmoud, D. R. Hill, and R. L. Guerrant for manuscript review, and Bonita Shannon for her assistance in manuscript preparation.

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In conjunction with the renewed interest in giardiasis in the past decade, there have been several epidemiologic studies implicating animals as potential sources of *Giardia* in human outbreaks [3, 4]. In the setting of waterborne outbreaks, beavers have been found to be infected with *Giardia* species that were infectious for specific pathogen-free beagles [3]. Furthermore, Davies and Hibler [4] demonstrated *Giardia* cysts that were indistinguishable from *G. lamblia* in the stool of a large number of wild and domestic animals. In the present study a common domestic animal, the mongrel dog, was investigated for its susceptibility to infection with cysts or trophozoites of *G. lamblia*.

Materials and Methods

Experimental animals. Mongrel dogs were obtained from the Cuyahoga County Animal Shelter, Cleveland, and were housed in separate cages in the Animal Resource Center of Case Western Reserve University School of Medicine, Cleveland. Two of the dogs, no. 3 and no. 4, were clearly puppies of less than six months of age, whereas the others were mature. During the course of study, dogs were maintained on a regular diet of Purina lab chow (Ralston Purina Co., St. Louis) and were weighed weekly; feedings were not discontinued at any time during the studies. Before inclusion in the study, a minimum of six

stool specimens from each animal was examined for *Giardia* cysts per week for at least three weeks by the sucrose flotation method described by Roberts-Thomson et al. [5]. Approximately 1 g of stool was processed for each examination. Animals with any *Giardia* cysts detected were excluded from the study. Of 37 dogs screened, 68% were found to be infected with *Giardia* species and were thus excluded. Because the speciation of *Giardia* is not defined by criteria other than the organism's host of origin and morphology, it was not possible to determine whether the excluded animals were infected with *G. lamblia*. Stools were also examined for other intestinal parasites, and the presence of parasites in stools was recorded. Only two study animals (no. 5 and no. 6) were infected with other parasites; both animals were excreting cysts of *Isospora belli*.

Preparation and inoculation of G. lamblia. *G. lamblia* cysts were obtained from stool specimens obtained from patients with symptomatic giardiasis or from persons who were chronic cyst excretors. After a stool specimen was obtained and the presence of *Giardia* cysts was confirmed by observation of wet preparations of stool, the specimen was dispersed in 0.95% NaCl and filtered through four layers of gauze. The number of cysts in the resultant suspension was determined, and the cysts were administered orally to one or two dogs. All cysts were used within 24 hr of collection.

Three dogs were given trophozoites of *G. lamblia* obtained from an in vitro axenic culture. The Portland-1 strain of *G. lamblia* trophozoites was grown in Diamond's medium as modified by Visvesvara [6], except that fetal calf serum rather than horse serum was used [6]. Trophozoites (1.5×10^6) were administered in 50 ml of culture medium to the three dogs. The culture medium contained considerable buffering capacity by virtue of its protein and serum components.

Characterization of G. lamblia infection. Study animals were followed with daily stool examinations. When *Giardia* cysts were detected, their number was determined and expressed as the number of cysts/g of stool (wet weight). Stool examinations were continued until clearance of cyst excretion (absence of cysts for more than three weeks) or for a minimum of 30 days. Two inoculated animals that did not become infected were killed, and their duodenal contents were examined directly for *Giardia*.

Although the animals were housed in separate cages, it was necessary to establish controls to determine the possibility of transmission between cages and to determine whether (or when) latent infection became patent. For each pair of dogs inoculated with *Giardia* cysts (and for the three dogs inoculated with trophozoites), an uninfected dog served as an uninoculated control. These five control animals were followed with the same daily stool examinations as were inoculated animals for the duration of the observation period. At no time did the control animals become infected.

Cysts excreted by experimentally infected animals were examined morphologically and were indistinguishable from the *G. lamblia* cysts that were used for inoculation.

Results

Inoculation with cysts. Three (37.5%) of eight dogs given *G. lamblia* cysts orally became infected (table 1). The mean latency period before cyst excretion was 8.3 days, with a range of five to 12 days. Two of the three dogs (no. 5 and no. 8) appeared to clear their cyst excretion after 6 and 2.5 weeks, respectively, whereas the third dog (no. 2) still had demonstrable cysts in its stool after almost 24 weeks. The two puppies (no. 3 and no. 4) were given 10^6 cysts and did not become infected. Two dogs that did not become infected after inoculation (no. 1 and no. 7) were killed, and no *Giardia* cysts were detected in their duodenal contents.

Inoculation with trophozoites. Three dogs were inoculated orally with 1.5×10^6 *G. lamblia* trophozoites (Portland-1 strain) from an in vitro culture (table 1). Two of the three animals became infected. As with the cyst-inoculated group, cyst excretion became manifest with latency periods of five and eight days. Both animals continued their cyst excretion for >30 days.

All infected animals (whether infected by cyst or by trophozoite) demonstrated cyclic cyst excretion, an example of which is shown in figure 1. The mean cycle length (for all infected animals) was 11.6 ± 0.9 days. (Cycle length was determined by calculating the number of days between peaks of cyst excretion. A peak of cyst excretion was defined as detectable cyst excretion for two or more consecutive days.) The maximum cyst counts ranged from 0.003×10^6 to 1.06×10^6 cysts/g of stool. Because daily stool output was ~60–200 g, each animal—with the exception of dog no. 8—had a

Table 1. Characteristics of mongrel dogs experimentally infected with *Giardia lamblia*.

Dog no. (sex)	*G. lamblia*			Outcome	Latency period (days)	Duration of cyst excretion (days)	Maximum cyst count (no. of cysts/ g of stool)
	Source	Form	Dose				
1 (M)	Symptomatic human adult	Cysts	5×10^5	Negative	. . .	. . .	. . .
2 (F)	Asymptomatic human adult	Cysts	1.2×10^6	Infection	8	>166	1.06×10^6
3 (M)	Asymptomatic human adult	Cysts	10^6	Negative	. . .	. . .	. . .
4 (F)	Asymptomatic human adult	Cysts	10^6	Negative	. . .	. . .	. . .
5 (M)	Asymptomatic human adult	Cysts	1.2×10^6	Infection	5	42	0.19×10^6
6 (F)	Symptomatic human adult	Cysts	10^6	Negative	. . .	. . .	. . .
7 (F)	Asymptomatic human adult	Cysts	10^6	Negative	. . .	. . .	. . .
8 (F)	Symptomatic human child	Cysts	3.2×10^6	Infection	12	17	0.003×10^6
9 (M)	Axenic culture	Trophozoites	1.5×10^6	Negative	. . .	. . .	. . .
10 (F)	Axenic culture	Trophozoites	1.5×10^6	Infection	5	>30	0.13×10^6
11 (M)	Axenic culture	Trophozoites	1.5×10^6	Infection	8	>30	0.08×10^6

NOTE. For a description of the methods used to prepare and inoculate *G. lamblia* organisms, see Materials and Methods.

regular daily cyst output that exceeded the original inoculum. It is of interest that the dogs with the longest and shortest durations of cyst excretion (no. 2 and no. 8, respectively) had the highest and lowest maximum cyst counts, respectively.

During the entire study course, all animals maintained their weights. During several periods, stool consistency was graded for control and infected animals. There was no correlation between looseness of stool and presence of *Giardia* infection or level of cyst excretion.

Discussion

The debate over interspecies transmission of *Giardia*, especially between humans and other mammals, has been intermittently active for >60 years. Although the early work of Hegner [1] suggested rigid host specificity for several *Giardia* species, he later demonstrated infection of rats with *Giardia* cysts from humans [1A]. Soon thereafter, Kessel [7] reported the apparent infection of eight of 15 kittens and one of three puppies with *G. lamblia*; however, there was no information given on the source or dose of the infecting parasite or on experimental controls. In 1969, Padchenko and Stolyarchuk [8] described experiments in which naturally acquired *Giardia canis* spread through a group of puppies housed together. In addition, Padchenko and Stolyarchuk administered *G. lamblia* cysts obtained from children to puppies that were apparently free from natural infection. The puppies became infected with latency periods of five to 29 days, and some developed intestinal disorders but had normal mucosa on histologic examination [8]. More recently, Vinayak et al. [9] reported infection of Swiss weanling mice wi.' *G. lamblia* cysts obtained from humans.

In a large study using a wide variety of experimental animals, Davies and Hibler [4] found that some animals (rats, gerbils, guinea pigs, beavers,

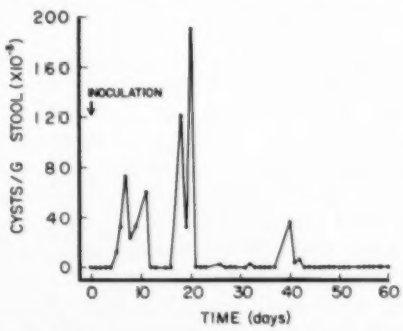

Figure 1. Cyclic cyst excretion by a dog (no. 5) that was orally inoculated with 1.2×10^6 *Giardia lamblia* cysts from human stool. For a description of the methods used to prepare and inoculate *G. lamblia* cysts, see Materials and Methods.

dogs, racoons, and bighorn sheep) but not others (hamsters, mice, rabbits, deer, and domestic sheep and cattle) were able to be infected with *Giardia* cysts from humans [4]. The duration of infection and clinical manifestations were not discussed. In a separate part of the study, fecal specimens from a variety of wild and domestic animals in Colorado were examined and found to contain *Giardia* cysts with an overall incidence of 9%.

In the present study, we have presented data to support the general concept of interspecies transmission of *Giardia*. Mongrel dogs were infected with cysts or trophozoites at an overall rate of 45%. Cyst excretion began with a mean latency period of 7.6 days after inoculation and demonstrated a cyclic pattern (figure 1). This pattern is strikingly similar to that observed by Rendtorff in his classic description of experimental giardiasis in prison volunteers [10].

The use of mongrel dogs obviously introduces the possibility that the experimental subjects might have had prior exposure to or infection with *Giardia*. Indeed, studies have demonstrated that the incidence of giardial infection in dogs ranges from 7.7% to 17.7% [11, 12]. We believe, however, that several factors provide strong support for the conclusion that these dogs were successfully infected experimentally rather than reactivating a latent, naturally acquired infection. First, the animals were carefully screened for a minimum of three weeks, and the control animals never developed cyst excretion. Second, in all infected animals cyst excretion began within a relatively narrow range of latency periods (five to 12 days). Finally, the excreted cysts were morphologically indistinguishable from the *G. lamblia* cysts that were used for inoculation.

There are several variables that may have contributed to the overall infection rate of <50%. As has been shown with *G. muris* infection in the mouse model, prior exposure or infection may confer some degree of resistance to subsequent challenge with the parasite [5]. On the other hand, young animals were not found to be more susceptible in that the only two puppies (less than one year of age) in the study (no. 3 and no. 4) were not infected by *Giardia* cysts. The animals that became infected were all fully mature. Finally, it is possible that there are significant differences in the infectivity of cysts from various patients or from the same patient on different days. Although Bingham and Meyer [13] have demonstrated in vitro excystation after acid treatment, it is not known whether the ease of excystation is a reliable indicator for infectivity. Therefore, at present, infectivity of cysts is a variable for which there is no independent marker. By the use of cultured trophozoites rather than cysts, we eliminated the contributions of unknown donors and recipient host factors as well as the need for excystation. This factor may account for the infection rate of 66.7% in dogs receiving cultured trophozoites in the well-buffered medium in contrast to 37.5% in those receiving cysts. Although the trophozoite is not thought to be the usual form of transmission, it is important to recognize that infection by this form is possible. Experimental infection of mice by single trophozoites of *G. muris* has been reported [14]. The present data represent the first report of infection in a mammal by trophozoites of *G. lamblia* (previously, *G. duodenalis*).

There are growing epidemiologic data to support the potential significance of wild and domestic animals as a reservoir for *Giardia*. Beavers with *Giardia* were found in an outbreak in the watershed area of Camas, Wash. [3]. *Giardia* cysts from those beavers and from filtration sediment obtained in another outbreak were found to be infectious for specific pathogen-free beagles [3]. A striking example of apparent interspecies transmission occurred at a zoo in Kansas City [15]. An outbreak of giardiasis involved three zoo attendants, two gibbons, two chimpanzees, a gorilla, and an orangutan. Transmission was interrupted by changes in the personal hygiene procedures of the staff. Further studies are required to determine whether similar outbreaks occur with direct transmission between humans and dogs.

We have presented data indicating that dogs can be infected with *G. lamblia* cysts or axenically cultured trophozoites from humans. We believe that the accumulating information refutes the hypothesis of rigid species specificity and supports the concept of a wide host range for *G. lamblia*. Recognition of the potential for transmission of *G. lamblia* from humans to dogs (and possibly back to humans) may be important clinically. The successful infection of 45% of the dogs indicates that (*1*) infection with *G. lamblia* is not restricted to humans; (*2*) the trophozoite form, although not long viable in nature, can be infectious; and (*3*) dogs as household pets should be considered as

a possible source or transmission link in the spread of giardiasis in the home. In light of these results, a patient with giardiasis that is not related to travel should be questioned about household pets, and consideration should be given to stool examination of the domestic animals.

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